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BODY WATER COMPARTMENTS DURING BED REST: EVALUATION OF ANALYTICAL METHODS

by H. L. Young, L. Juhos, B. L. Castle, J. Yusken, and J. E. Greenleaf

Ames Research Center
Moffett Field, Calif. 94035

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16. Abstract

Nine healthy young men, ages 21 to 25, were studied to determine the reproducibility and interchangeability of the use of (a) radio-iodinated human serum albumin (I^{131}) and Evans Blue dye for estimating plasma volume, (b) sodium bromide for extracellular fluid volume, and (c) deuterium oxide (D_2O) for total body water volume. All subjects were tested in a semibasal condition and allowed to rest for at least 30 min after arriving at the laboratory. Evans Blue and I^{131} were administered intravenously and NaBr and D_2O by oral ingestion. Venous blood samples were collected for analysis at various intervals after the tracers were administered. For the measurement of plasma volume, a comparison was made between the 10-min postinjection blood sample and the 0-min value extrapolated from the 10-, 20-, 30-, and 60-min samples. For NaBr and D_2O spaces, a comparison was made between the three 3-hr postingestion blood samples and the 0-min value extrapolated from the 2-, 3-, 5-, and 8-hr samples. The results indicate (a) there was uniform distribution of I^{131} and Evans Blue dye 10 min after injection and of NaBr and D_2O 3 hours after oral ingestion; (b) the buildup of residual tracer did not interfere appreciably with the measurement of either I^{131} or Evans Blue spaces when they were administered at four equal intervals over 8 days or three times in 15 days; and (c) the buildup of background tracer after ingestion of NaBr and D_2O once per week for three consecutive weeks did not affect the accuracy of the measurement. Average interindividual variability (±SE) over a 3-week period was ±155 ml for I^{131} space, ±146 ml for Evans Blue space, ±310 ml for bromide space, and ±2,300 ml for total body water space. It was found that (a) I^{131} and Evans Blue may be used interchangeably for estimating plasma volume and one 10-min equilibration sample gives results comparable to those obtained using the 0-min extrapolation of multiple blood samples, and (b) for estimating bromide and D_2O spaces, one 3-hr equilibrium blood sample gives results similar to the extrapolation of multiple samples.

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Ames Research Center

SUMMARY

Nine healthy young men, ages 21 to 25, were studied to determine the reproducibility and interchangeability of the use of (a) radio-iodinated human serum albumin (I^{131}) and Evans Blue dye for estimating plasma volume, (b) sodium bromide for extracellular fluid volume, and (c) deuterium oxide (D_2O) for total body water volume. All subjects were tested in a semibasal condition and allowed to rest for at least 30 min after arriving at the laboratory. Evans Blue and I^{131} were administered intravenously and NaBr and D_2O by oral ingestion. Venous blood samples were collected for analysis at various intervals after the tracers were administered. For the measurement of plasma volume, a comparison was made between the 10-min postinjection blood sample and the 0-min value extrapolated from the 10-, 20-, 30-, and 60-min samples. For NaBr and D_2O spaces, a comparison was made between the three 3-hr postingestion blood samples and the 0-min value extrapolated from the 2-, 3-, 5-, and 8-hr samples. The results indicate (a) there was uniform distribution of I^{131} and Evans Blue dye 10 min after injection and of NaBr and D_2O 3 hours after oral ingestion; (b) the buildup of residual tracer did not interfere appreciably with the measurement of either I^{131} or Evans Blue spaces when they were administered at four equal intervals over 8 days or three times in 15 days; and (c) the buildup of background tracer after ingestion of NaBr and D_2O once per week for three consecutive weeks did not affect the accuracy of the measurement. Average interindividual variability (±SE) over a 3-week period was ±155 ml for I^{131} space, ±146 ml for Evans Blue space, ±310 ml for bromide space, and ±2,300 ml for total body water space. It was found that (a) I^{131} and Evans Blue may be used interchangeably for estimating plasma volume and one 10-min equilibration sample gives results comparable to those obtained using the 0-min extrapolation of multiple blood samples, and (b) for estimating bromide and D_2O spaces, one 3-hr equilibrium blood sample gives results similar to the extrapolation of multiple samples.

INTRODUCTION

Loss of total water and reduction in plasma volume have been reported in astronauts who have returned from spaceflight and also in subjects during prolonged bed rest. The mechanism of these changes is unknown, but it appears to result from reduced hydrostatic pressure in the circulatory system. To understand this mechanism, the accuracy of various analytical techniques for determining body fluid compartments must first be investigated.

Numerous analytical methods have been developed to measure the volumes of plasma (PV), extracellular fluid (ECF), and total body water (TBW) in man. All these methods are based on the tracer dilution principle. However, the results from different techniques for measuring any one fluid

compartment show some discrepancies, due partly to the inherent errors of the chemical analysis but mostly to the differences in distribution of the various tracers in the body.

To obtain simultaneous, accurate measurements of the changes in plasma volume, extracellular fluid volume, and total body water volume, the analytical methods should not interfere with each other and should fulfill the following general requirements: The tracer should be clinically acceptable for human use; it should be chemically stable, nontoxic, and relatively well characterized metabolically and physiologically; and it should be measurable with high accuracy and precision. The samples collected after the tracer(s) is administered should be easily accessible and convenient to analyze either immediately after withdrawal or after storage. In addition, the radioactive tracer should have a low radiation energy to minimize health hazards and a short biological half-life to afford multiple repeated measurements without a buildup of radiation, and there should be no loss in measurement accuracy while the sample-to-background ratio decreases to less than 2:1.

The selection of a method for measuring total body water is comparatively simple. The nonradioactive isotope, deuterium oxide, provides the advantages of an isotopic method, with good analytical accuracy but without the health hazard of radioactivity. Thus, the method using deuterium oxide fulfills the above requirements, but the analytical procedure is more tedious than when radioactive tritium is used (refs. 1 and 2).

Selecting a tracer for the determination of extracellular fluid volume is more difficult. To date, no single tracer is accepted unequivocally as ideally suited for this measurement. Substances commonly used to measure ECF may be classified into two groups of compounds (refs. 3–5). The first group of compounds (thiocyanates and bromides) estimates the volume occupied by the chloride ions in the body. The volume measured by these compounds is somewhat greater than the true extracellular space since the thiocyanate and bromide ions remain in the gastrointestinal tract and also enter the red blood cells (ref. 4). The second group of substances commonly used for this purpose include inulin, sucrose, thiosulfate, and sulfate. These substances do not remain in the gastrointestinal tract and do not penetrate the connective tissues. Thus, the "true" extracellular fluid volume probably lies between the bromide and inulin spaces (ref. 6). For the present study, the bromide space was selected for estimating extracellular fluid volume because both radioactive and nonradioactive bromides were available as tracers and because the analytical procedure for determining nonradioactive bromide is relatively simple and accurate. The short half-life of Br^{82} (36 hours) and its simple counting technique make it an attractive choice for future studies.

Two tracers are most commonly used for measuring plasma volume: radio-iodinated human serum albumin (I^{131}) and the azo-dye Evans Blue (T-1824) (refs. 7–11). Both substances are preferentially bound to plasma albumin and distributed within the plasma albumin space. Simultaneous measurement of PV with these two tracers has shown comparable results (refs. 8 and 9).

In the present study, I^{131} and Evans Blue were chosen to determine the plasma volume; sodium bromide and deuterium oxide were selected to measure the volumes of extracellular fluid and total body water, respectively. Although these tracers have often been used by different investigators, detailed information on the influence of one tracer on another during the simultaneous measurements of three water compartments, and on the effect of tracer accumulation on the subsequent determination is unavailable but necessary to obtain accurate and reproducible results on repeated measurements of body fluids. Therefore, the purpose of this study is to compare the interchangeability of I^{131} with Evans Blue for measuring plasma volume; to determine the

reproducibility of bromide and deuterium oxide measurements of ECF and TBW spaces, respectively; to determine the effect of I^{131} buildup on the measurement of Evans Blue space; and to determine the effect of bromide and deuterium oxide buildup on the measurement of ECF and TBW spaces, respectively.

PROCEDURE AND METHODS

Nine healthy men, ages 21 to 25, were instructed to abstain from food after 10 PM the night before the scheduled determination but without restriction of water intake. They arrived at the laboratory at 8 AM, were then weighed and allowed to rest in bed for 30 min. A urine sample was collected and a 10-ml preinjection control blood sample was taken without stasis from the antecubital vein with a 21 guage (21G) needle. The subjects remained in the horizontal position during the entire 2-hr period of the plasma volume measurement and during the first 3 hours of the extracellular fluid volume and the total body water measurements. After the 3-hr blood sample was withdrawn for the measurements of ECF and TBW, the subjects were allowed to sit up in bed. Food was withheld from the subjects during the 8-hr test, but 300 ml of water was given to provide sufficient urinary volume for accurate isotope measurements.

Plasma Volume

The plasma volume was determined by either one or both tracer methods with I^{131}[a] or Evans Blue dye.[b] All subjects participating in the plasma volume experiments were required to take 10 drops of Lugols solution in water daily 3 days before and also on the day of measurement to block the uptake of I^{131} by the thyroid gland.

The radioactive iodine technique was similar to that of Crispell, Porter, and Nieset (ref. 11) with the following modifications: A zero time blood sample was drawn with a 21G needle, and through the same needle approximately 1.5 ml of I^{131} (specific activity 5.0 μCi per 15 mg serum albumin in 0.45 percent sodium chloride, Lot #L812031) was injected. The syringe containing I^{131} and the needle were carefully weighed before the injection. After the injection of I^{131}, the needle was wiped clean and the syringe and needle were weighed again immediately. The exact amount of I^{131} injected was determined by the difference in weight of the syringe and needle before and after the injection. Blood samples were taken from the arm opposite that of the injection 10, 20, 30, 60, 90, and 120 min after injection. After the needle was disconnected, the blood sample was delivered slowly from the syringe into a sodium-heparinized glass tube and was mixed continuously on a mechanical rotator for approximately 5 min. The plasma was separated from the red cells by centrifugation (at approximately 800 G for 10 min) and 2 ml of plasma was transferred to a counting tube. The radioactivity of the plasma was counted in a Packard Tri-Carb series 314E liquid scintillation gamma spectrometer. Several I^{131} standards were prepared daily by diluting a weighed amount of commercial I^{131} solution in 500 ml of 0.9 percent sodium chloride solution. Two-ml volumes of 0.9 percent sodium chloride solution (as the background) and of the diluted standard were counted sequentially with the plasma samples. The apparent plasma volume (PV_a) was computed from the following equation:

[a] I^{131} (in sterilized syringes) was purchased from the Ames Division of Miles Laboratories Inc., Elkhart, Indiana.
[b] Evans Blue was manufactured by General Diagnostics Div., Warner-Chilcott, Morris Plains, N. Y. (5 ml).

$$PV_a(ml) = \left(\frac{A_S - A_{bk}}{A_p - A_{bk}}\right)\left(\frac{\text{weight of injected dose, gm}}{\text{weight of standard, gm}}\right)(500\ ml) \quad (1)$$

where A_S is the I^{131} radioactivity in the diluted standard, CPM/2 ml; A_{bk}, the background radioactivity, CPM/2 ml; and A_p the I^{131} radioactivity in plasma, CPM/2 ml. The actual plasma volume was determined by either: (a) the apparent plasma volume measured 10 min after I^{131} injection was taken as the actual plasma volume or (b) a straight-line fit computed by the method of least squares using all apparent plasma volumes measured within 2 hours (10, 20, 30, 60, 90, and 120 min). This line was extrapolated to zero time and the extrapolated value (the value a in eq. (2)) was taken as the actual plasma volume. The equation used for the least-squares fit was

$$\Sigma (Y - \bar{Y})^2 = \Sigma[Y - (a + bX)]^2 \quad (2)$$

where Y is the apparent plasma volume at any time; $\bar{Y}$, the corresponding predicted plasma volume; X, measurement time; a, the predicted plasma volume at zero time; and b, the slope of the straight line.

When Evans Blue is injected, it becomes rapidly and preferentially bound to the plasma albumin. Approximately 5 ml of a 0.5 percent aqueous dye solution was administered intravenously without further dilution. The exact dose administered, blood sampling, subject management, test procedure, and preparation of plasma were the same as for the I^{131} method. The treatment of the plasma samples, the determination of their Evans Blue dye concentration, and the subsequent computation of plasma volume were performed in a way similar to that of O'Brien, Ibbot, and Rodgerson (ref. 12). The procedure is as follows: (a) The Evans Blue stock standard was prepared by diluting 1 ml of the commercial (0.5 percent) stock dye preparation to 50 ml with distilled water. (b) The internal standard was prepared with 0.2 ml of Evans Blue diluted standard solution mixed with 2 ml of control plasma obtained prior to the injection of the dye. The quantity of Evans Blue recovered from the plasma mixture was analyzed spectrophotometrically and served as the internal standard. (c) The internal standard and plasma samples were mixed thoroughly with 15 ml of Teepol-phosphate, a detergent that displaces the dye from protein. The mixture was transferred gently onto a pulp column prepared with Solka-Floc SW-40A (Brown Co., Boston, Mass.). The column was rinsed with 5 ml of Teepol phosphate. Interfering substances such as protein, pigments, and chylomicrons were washed from the column with 2 percent disodium hydrogen phosphate. With this technique, the interference due to hemolysis can be minimized or eliminated completely. The flow rate of the eluate in the chromatographic columns was regulated with a stopcock to about one drop every 2 seconds. The dye was then eluted from the column with a freshly prepared alkaline acetone-water mixture (1:1) without adjustment of pH. (d) The light absorption of the plasma blanks, standards and the dye recovered from plasma were measured at 615 $m\mu$ with a Zeiss PMQ II spectrophotometer. (e) The plasma volume was computed from the following equation:

$$PV(ml) = \frac{EB_I \times \epsilon_{std} \times V}{\epsilon_{pl} \times EB_{std} \times 1.03} \quad (3)$$

where EB_I is the weight of Evans Blue injected (gm); ϵ_{std}, the absorbance of Evans Blue extracted from the plasma-standard mixture; V, the volume of plasma used for determination (ml); ϵ_{pl}, the absorbance of Evans Blue extracted from plasma; EB_{std}, the amount of Evans Blue added to the internal standard (ml of Evans Blue solution X 1/50 X 0.2); and 1.03, the correction for the estimated 3 percent Evans Blue absorbed by the tissues.

Total Body Water and Extracellular Fluid Volumes

The total body water and extracellular fluid volumes were measured simultaneously. After the initial urine and blood collections, approximately 50 ml of a sodium bromide-D_2O mixture (2.9 gm of NaBr in 49 gm of D_2O)[c] was administered orally using a syringe. The exact dose administered was determined by the difference in weight of the syringe before and after the NaBr-D_2O administration. Immediately after ingestion and 2 and 7 hours after ingestion of the NaBr-D_2O mixture, 100 ml of tap water was consumed. Exactly 2, 3, 5, and 8 hours after the ingestion of the tracers, 10-ml blood samples were drawn and processed as described under Plasma Volume. Urine samples were collected before ingestion and 3 and 8 hours after ingestion of the NaBr-D_2O mixture. All samples of plasma and urine were kept frozen at -6° C. Chemical analyses and computations of total body water and extracellular fluid volumes are described below.

Total body water (D_2O space) was determined from the administered dose of D_2O and the plasma concentration after equilibration in the body. Deuterium oxide in the plasma and urine samples was recovered by lyophilization and analyzed with a Beckman IR-9 infrared spectrophotometer (ref. 13). Matched calcium fluoride cells with 0.9 mm light paths were used for all measurements. Light absorption of each sample at 2,513 cm^{-1} was determined approximately 5 min after the cell was placed in the spectrophotometer to allow for temperature equilibration.

A D_2O standard curve was constructed with seven standards ranging from 0.01 to 0.2 ml D_2O per 100 ml of water and the light absorption was read at 2513 cm^{-1} against distilled water. The linear curve was fitted to the data by the method of least squares. The result of D_2O analyses showed that 97.3 to 103.0 percent (mean of 99.7 percent) of the standard added to plasma in vitro was recovered. The total body water at each period of measurement was computed as follows: (a) The weight of NaBr was subtracted from the weight of NaBr-D_2O mixture administered. (b) The D_2O administered was converted from gravimetric (gm) to volumetric units (ml) from:

$$D_2O \text{ ingested (ml)} = \frac{\text{weight of } D_2O \times 0.9976}{1.104} \tag{4}$$

where 0.9976 is the purity of D_2O and 1.104 the density of D_2O at 22° C. (c) The volume of D_2O excreted in urine was calculated from:

$$D_2O \text{ excreted (ml)} = \frac{OD_u \times [D_2O]_{std} \times V_u}{OD_{std}} \tag{5}$$

[c]NaBr-D_2O solution: 1.5 gm of NaBr in 25 gm of D_2O, sterile and pyrogen free, was manufactured by Merck and Co., Inc., Canada.

where OD_u and OD_{std} are the light absorptions of urine and the standard at 2,513 cm^{-1}; $[D_2O]_{std}$ is the concentration of D_2O of the standard (in volume percent); and Vu is the total volume of urine excreted (in ml). (d) The concentration of D_2O in plasma $[D_2O]_{pl}$ (in volume percent) was calculated from:

$$[D_2O]_{pl} = \frac{OD_{pl} \times [D_2O]_{std}}{OD_{std}} \tag{6}$$

(e) The total body water (in liters) was computed from:

$$\text{Total body water (liters)} = \frac{D_2O_{\text{ingested}} - D_2O_{\text{excreted}}}{[D_2O]_{pl} \times 10} \tag{7}$$

(f) The total body water given in the present study was computed from the single measurement of D_2O concentration in plasma 3 hours after ingestion, and from the serial measurements 2, 3, 5, and 8 hours extrapolated to zero time after D_2O administration, with the curve fitted by the method of least squares.

Extracellular fluid volume (bromide space) was determined from the plasma concentration of bromide at equilibrium after the ingestion of a known quantity of NaBr. The bromide concentration in plasma was determined by the microdiffusion technique of Conway (ref. 14) as modified by Cheek (ref. 15). One ml of plasma was placed in a 15-ml clean glass-stoppered tube and, after 1 ml of distilled water was added, the contents of the tube were dried in an oven at 95° C. Five ml of distilled water was added to the dry residue and agitated with a Vortex mixer for 1 hour. The tubes were then centrifuged and a 4-ml aliquot of the clear supernatant was pipetted to the outer chamber of a pyrex Conway microdiffusion cell. The supernatant was dried at 95° C in the Conway cell and then ashed at 400° C for 20 min. After cooling, approximately 0.2 gm of potassium dichromate was added to the outer chamber and 1 ml of 20 percent potassium iodide was added to the inner chamber. The cell was half closed with a lid lubricated with silicone grease (Dow Corning); after 1 ml of 40 percent sulphuric acid was added to the outer chamber, the cell was closed completely. The contents of the outer chamber were mixed by rotation. The Conway cell remained at room temperature for 2 hours so that the complete diffusion of bromide would replace the iodide in the inner chamber. The liberated iodide in the inner chamber was then titrated with 0.01 N sodium thiosulfate from a microburette (Manostat Corp., N.Y.), with 0.2 percent starch solution as the end-point indicator.

Sodium bromide standards, ranging from 0.01 to 2.0 mEq/liter, were prepared with plasma and analyzed with the same procedure as for the samples. The result of the Br analyses showed that 92.5 to 97.5 percent (mean of 95.0 percent) of the standard added to plasma in vitro was recovered. A standard curve was constructed from the bromide standards. Bromide space was computed from:

$$\text{Bromide space (liters)} = \frac{Br_I \times 0.9 \times 0.95 \times 0.94}{[Br]_{pl}} \tag{8}$$

where Br_I is the ingested bromide (in mEq); 0.9, the proportion of the bromide remaining in the plasma (10 percent of the bromide is assumed to enter the red blood cells); 0.95, the Donnan equilibrium factor; 0.94, the density of the plasma; and $[Br]_{pl}$, the plasma bromide concentration.

Results were analyzed with the appropriate t-test and the level of significance was $\alpha = 0.05$. (Nonsignificant differences are indicated by NS.)

RESULTS AND DISCUSSION

Plasma Volume

The I^{131} albumin spaces measured on nine subjects are shown in table 1. The mean albumin space measured 10 min after the tracer was administered was 3,347 ml and that obtained from the

TABLE 1.- COMPARISON OF PLASMA VOLUMES USING I^{131} AND EVANS BLUE BETWEEN ONE 10-MIN POSTINJECTION SAMPLE AND THE ZERO TIME EXTRAPOLATED VALUE

I^{131} Space					Evans Blue space				
Subject	N	0 min (ml)	10 min (ml)	$\frac{0\ min}{10\ min} \times 100$	Subject	N	0 min (ml)	10 min (ml)	$\frac{0\ min}{10\ min} \times 100$
NP	5	3368	3417	98.6	NP	5	3133	3167	98.9
TA	5	2906	2930	99.2	TA	5	2661	2674	99.5
DL	1	4586	4590	99.9	DL	1	3194	3306	96.6
RK	5	3310	3194	103.6	RK	5	2835	2837	99.9
AO	5	3353	3367	99.6	AO	5	3011	3064	98.3
RR	1	3114	3085	100.9	TS	3	3106	3059	101.5
VC	1	3015	3014	100.0	JRo	3	4103	3898	105.3
RS	1	3629	3664	99.0	VC	3	2925	2962	98.8
RR	1	2854	2864	99.7	KN	3	2862	2913	98.2
					CH	3	3633	3608	100.7
					JRe	3	4400	4355	101.0
					TV	3	2753	2841	96.9
Mean		3369	3347	100.1	Mean		3218	3224	99.6
±SE		175	177	.50	±SE		158	143	.68

extrapolated slope of four measurements during the first hour was 3,369 ml (table 1). The individual variations of I^{131} spaces in four measurements over an 8-day period were very small; the means (±SE) were 3,317 ±60 ml for NP, 2,673 ±45 ml for TA, 2,822 ±70 ml for RK, and 3,363 ±165 ml for AO. The mean percentage difference between the 0-min and the 10-min calculation was 0.1 percent (NS, fig. 1). These results indicate that the short method using the 10-min interval after I^{131} is administered is comparable to the extrapolation method involving serial measurements.

Evans Blue space volumes were compared at 10-min postinjection versus the extrapolation method during the first hour after the tracer was administered (table 1). The individual variations of Evans Blue spaces in four measurements over an 8-day period were similar to the I^{131} spaces; the means (±SE) were 3,232 (±101) ml for NP, 2,654 (±61) ml for TA, 2,766 (±85) ml for RK, and 3,142 (±66) ml for AO. The Evans Blue spaces determined by either method on 12 subjects were 3,218 ml for the extrapolated 0-min value and 3,224 ml for the 10-min measurements (NS, fig.2). The mean percentage difference between the 0-min extrapolation and the 10-min data was 0.4 percent (NS). Thus, Evans Blue space determined from a single sample obtained 10 min after the tracer is administered is comparable to the zero extrapolation value from serial sampling.

A comparison was made between I^{131} space and Evans Blue space measured simultaneously (fig. 1 and table 1). In subjects NP and TA, the I^{131} determinations preceded the Evans Blue measurements, while in subjects RK and AO, Evans Blue space was measured first. Although not statistically significant, the I^{131} space was consistently larger than the Evans Blue space, varying from 1 to 7 percent measured by the 10-min technique (NP, 3 percent; TA, 1 percent; PK, 2 percent; AO, 7 percent). Schultz, Hammersten, Heller, and Ebert (ref. 16) and Seer, Allen, and Gregerson (ref. 9) found that Evans Blue gave slightly higher values (NS) than I^{131}. Their comparisons were made on the basis of single dye and isotope determination on each subject. Zipf, Webber, and Grove (ref. 8) compared these two tracers on 34 adult males from two independent blood samples drawn 15 to 20 min after injection. The mean plasma volume determined by Evans Blue (52.1 ml/kg) was 25.5 percent higher than that obtained with I^{131} (41.5 ml/kg). The Evans Blue method has a mean probable error of 213 ml (5.2 percent) while the I^{131} method yielded an average probable error of 57 ml (2.3 percent). Zipf et al. concluded that the difference in the results between the two methods was due to the differences in the inherent errors in the volumetric (Evans Blue) and gravimetric (I^{131}) analytical techniques and not to any intrinsic difference in the biochemical or physiologic characteristics of the two tracers. Crispell et al. (ref. 11) determined the plasma volume of 19 adult males using I^{131} albumin and Evans Blue dye simultaneously; the I^{131} space in 10 subjects was greater than the Evans Blue space and lower in the other 9 subjects. These differences were not statistically significant. In the present study I^{131} resulted in a slightly greater plasma volume than Evans Blue. Two possible explanations are worth mentioning: (a) The discrepancy may be due to the intrinsic difference in the biochemical or physiologic characteristics of the two substances or (b) some I^{131} in the commercial I^{131} RIHSA may be present as the free iodide and not tagged onto the serum albumin molecules; thus it could enter the interstitial space during the first 10 min of the measurement. Loss of tracer after injection would result in a larger calculated

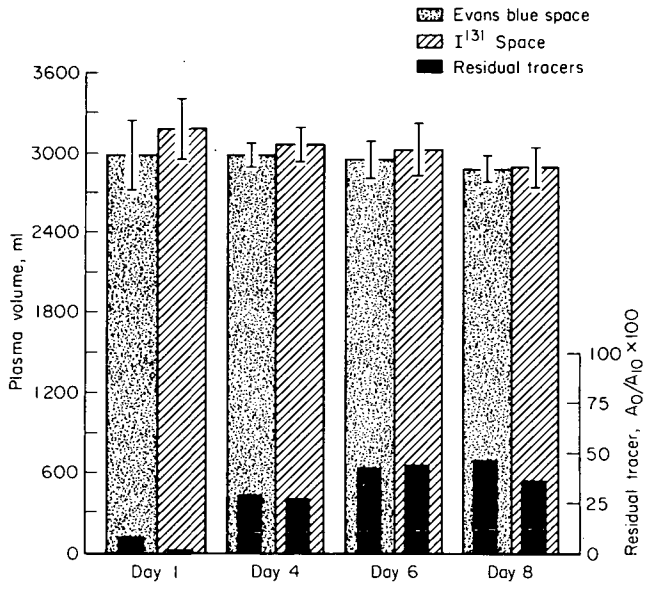


Figure 1.– Comparison of average I^{131} (striped bars) and Evans Blue spaces (mean ±SE) of simultaneous measurements on four subjects. The solid bars at the bottom represent the residual tracer (expressed as a percent of total tracer in the plasma after injection).

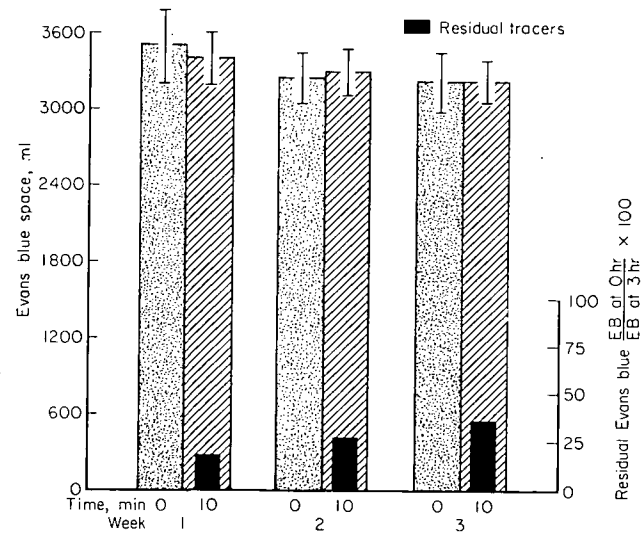


Figure 2.– Comparison of average Evans Blue spaces of seven subjects measured in three consecutive weeks between the 0-min extrapolation value and that computed from the 10-min sample. The solid bars at the bottom represent the residual Evans Blue (expressed as a percent of total dye after injection).

TABLE 2.– ACCUMULATION OF I^{131} RIHSA AND I^{131} RIHSA SPACE FROM REPEATED INJECTIONS

Group 1												
	Day 1			Day 4			Day 6			Day 8		
Subject	Residual I^{131} (CPM)	$\frac{A_0}{A_I}$ (100)*	I^{131} Space (ml)	Residual I^{131} (CPM)	$\frac{A_0}{A_I}$ (100)	I^{131} Space (ml)	Residual I^{131} (CPM)	$\frac{A_0}{A_I}$ (100)	I^{131} Space (ml)	Residual I^{131} (CPM)	$\frac{A_0}{A_I}$ (100)	I^{131} Space (ml)
NP	139	0.486	3427	6640	42.00	3150	11,740	85.35	3368	12.779	64.13	3322
TA	68	.258	2631	7722	41.94	2795	13,908	80.13	2588	14.684	60.53	2677
RK	50	.212	2978	7270	39.65	2886	12,932	77.06	2771	13.582	54.61	2653
AO	32	.168	3747	5720	38.64	3410	10,027	75.53	3352	10.853	49.14	2944
Mean		.281	3198		40.56	3060		79.52	3019		57.10	2899
±SE		.142	245.6		.84	138.8		2.17	200.0		3.30	155.7
Group 2												
	Day 1			Day 3			Day 5			Day 7		
RR	14	.069	2763	6854	39.96	2272	10,230	39.29	2850	16,320	74.68	2790
CH	1	.005	3130	5938	47.04	3774	8,915	38.69	3194	14,108	78.33	3598
Mean		.038	2947		43.50	3023		38.99	3022		76.51	3194

*A_0 is the I^{131} activity of plasma before injection (CPM/2 ml plasma); A_I is the I^{131} activity from injection (CPM/2 ml plasma) (plasma activity at 10-min - plasma activity at zero time).

plasma volume. Evans Blue has one advantage over I^{131} as a tracer in that Evans Blue will discolor the arm at the site of injection if any tracer escapes into the extravascular space.

The effect of I^{131} buildup on I^{131} space was determined in four subjects (group 1) who received I^{131} injections on days 1, 3, 5, and 7 (table 2). At the beginning of the fourth test (day 8), the residual I^{131} activity in the plasma varied from 3.5 to 4.3 μCi and after the fourth injection, it varied from 8.5 to 9.3 μCi. In group 1 the calculated mean (±SE) plasma volume, determined when the residual I^{131} was 75 to 85 percent of the injection dose on day 6, or 3,019 (±200) ml was essentially the same as the mean plasma volume on day 1 or 3,198 (±246) ml. The variability (±SE) of the mean plasma volume was lower on days 4, 6, and 8 than on day 1. In group 2, the day 7 mean plasma volume was 3,194 ml compared with 2,947 ml on day 1 (NS, table 2). When the residual radioactivity is accounted for, these results indicate that repeated injections of I^{131} every other day with a total of four injections do not affect the measurement of plasma volume significantly.

The effect of Evans Blue dye buildup on Evans Blue space was measured in four subjects on days 1, 4, 6, and 8 (group 1) and in seven subjects on days 1, 8, and 15 (group 2) (see table 3 and fig. 2). In group 1, the mean plasma volumes over the eight days varied from 2,979 ml on day 1 to 2,873 ml on day 8, a difference of 3.6 percent. In group 1, the residual Evans Blue concentration is about 41 percent of the injected dose on day 4, 75 percent on day 6, and 87 percent on day 8, but the Evans Blue spaces on day 6 and day 8 were only slightly lower than on day 1. A similar decrease over time was obtained when the plasma volume was measured with I^{131} (table 2). The decline of Evans Blue space possibly represents a change in plasma volume rather than the effect of Evans Blue accumulation.

The effect of Evans Blue buildup on the determination of plasma volume in seven subjects at weekly intervals (table 3, group 2) showed that Evans Blue space on day 15 (3,191 ml) was

TABLE 3.– ACCUMULATION OF EVANS BLUE AND EVANS BLUE SPACE IN REPEATED INJECTIONS

Group 1								
	Day 1				Day 4			
Subject	Residual Evans Blue $\epsilon_0$615 mμ	Injected dose ϵ_I615 mμ	$\frac{\epsilon_0}{\epsilon_I} \times 100$*	Evans Blue Space (ml)	Residual Evans Blue $\epsilon_0$615 mμ	Injected dose ϵ_I615 mμ	$\frac{\epsilon_0}{\epsilon_I} \times 100$	Evans Blue Space (ml)
NP	0.006	0.120	5.00	3532	0.062	0.122	50.82	3156
TA	.012	.169	7.10	2476	.066	.147	44.90	2734
RK	.015	.150	10.00	2600	.054	.141	38.30	3001
AO	.017	.117	14.53	3308	.041	.132	31.06	3066
Mean	.013	.139	9.16	2979	.056	.136	41.27	2990
±SE	.002	.012	2.06	260	.006	.005	4.26	92
	Day 6				Day 8			
NP	0.091	0.124	73.39	3151	0.121	0.132	91.67	3087
TA	.125	.140	89.29	2730	.140	.153	91.50	2674
RK	.104	.145	71.72	2742	.141	.151	93.38	2720
AO	.080	.120	66.67	3182	.101	.140	72.14	3011
Mean	.100	.132	75.27	2951	.126	.144	87.17	2873
±SE	.010	.006	4.89	124	.009	.005	5.03	103

Group 2												
	Day 1				Day 8				Day 15			
Subject	Residual Evans Blue $\epsilon_0$615 mμ	Injected dose ϵ_I615 mμ	$\frac{\epsilon_0}{\epsilon_I} \times 100$	Evans Blue Space (ml)	Residual Evans Blue $\epsilon_0$615 mμ	Injected dose ϵ_I615 mμ	$\frac{\epsilon_0}{\epsilon_I} \times 100$	Evans Blue Space (ml)	Residual Evans Blue $\epsilon_0$615 mμ	Injected dose ϵ_I615 mμ	$\frac{\epsilon_0}{\epsilon_I} \times 100$	Evans Blue Space (ml)
TS	0.012	0.109	11.01	3387	0.053	0.129	41.09	2779	0.046	0.140	32.86	3011
JRo	.014	.104	13.46	3735	.036	.166	21.69	4023	.039	.096	40.63	3937
VC	.018	.068	11.76	2906	.024	.134	17.91	3106	.044	.137	32.12	2875
KN	.012	.142	8.45	2898	.031	.130	23.85	2970	.043	.138	31.16	2872
CH	.008	.108	7.41	4079	.024	.097	24.74	3252	.033	.078	42.31	3495
JRe	.012	.069	17.39	3983	.026	.136	19.12	3901	–	–	–	–
TV	.017	.086	19.77	2848	.035	.122	28.69	2920	.051	.106	48.11	2756
Mean	.013	.098	12.75	3405	.033	.131	25.30	3279	.043	.116	37.87	3191
±SE	.001	.010	1.71	202	.004	.008	2.96	180	.003	.011	2.80	177

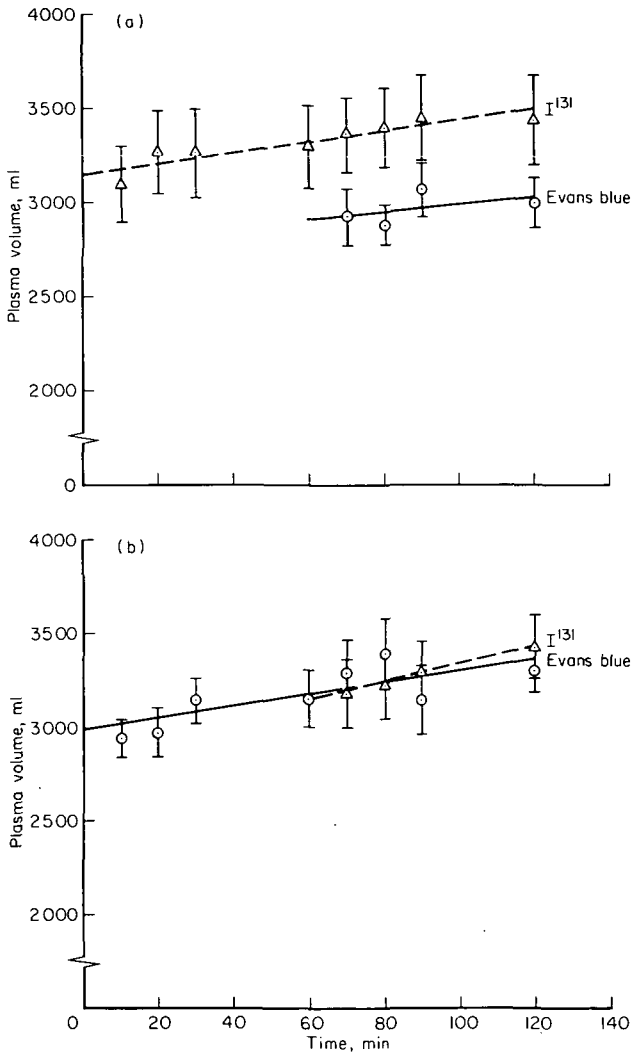
*ϵ_0 is the absorbance of Evans Blue extracted from 0-min plasma; ϵ_I is the absorbance of Evans Blue extracted from 10-min plasma – ϵ_0. Evans Blue Space was computed from 10-min plasma sample.

TABLE 4.– I^{131} SPACE AND EVANS BLUE SPACE OF YOUNG HEALTHY MALES

I^{131} Space							Evans Blue space						
Subject	N	Mean (ml)	±SD	±SE	ml/kg	liter/m²	Subject	N	Mean (ml)	±SD	±SE	ml/kg	liter/m²
NP	4	3198	188	94	43.1	1.68	NP	3	3167	333	192	42.7	1.67
TA	5	2834	219	98	42.0	1.57	TA	3	2585	101	58	38.4	1.43
DL	1	4590	-	-	44.4	2.01	DL	1	3306	-	-	32.0	1.45
RK	5	3048	389	174	42.0	1.64	RK	3	2837	314	181	39.1	1.53
AO	4	3064	295	132	32.9	1.44	AO	3	3064	222	128	32.9	1.44
CH	4	3425	311	156	42.7	1.67	CH	3	3608	425	245	44.9	1.76
RR	5	2852	135	60	41.9	1.58	TS	3	3059	307	177	42.8	1.67
RC	1	2610	-	-	46.6	-	JRo	3	3898	148	85	48.0	1.85
JR	1	3532	-	-	55.2	2.03	VC	3	2962	126	73	41.3	1.59
KS	1	3817	-	-	51.7	2.00	KN	3	2913	51	29	41.4	1.57
MT	2	4168	194	138	57.0	2.13	JRe	3	4355	716	413	50.5	2.09
TD	2	3330	28	20	47.7	1.79	TV	3	2841	82	48	40.3	1.56
RW	2	3141	16	11	52.5	-							
Mean		3355	197	98	46.1	1.78	Mean		3216	257	148	41.2	1.63
±SE		155			1.84	.07	±SE		146			1.55	.06

6.3 percent lower than on day 1 (3,405 ml), with the day 8 value in between. Again there is a decrease of Evans Blue space with time as in group 1. These results indicate that Evans Blue buildup does not interfere appreciably with the measurement of Evans Blue space when it is administered four times in 8 days or three times in 15 days.

To determine if Evans Blue interferes with the measurement of I^{131} space and the latter with Evans Blue space, one tracer was injected at zero time and four blood samples were than obtained 10, 20, 30, and 60 min thereafter. At 60 min, the second tracer was injected and four more blood samples were then withdrawn at 70, 80, 90, and 120 min. The slope of the 0- to 60-min tracer measurements was compared with the slope of the second tracer measurements. The two slopes were very similar regardless of which tracer was injected first (fig. 3). Thus, it is concluded that the Evans Blue and I^{131} do not interfere with each other and the two substances may be used interchangeably for daily plasma volume determination.



(a) Each point represents the mean (±SE) of three tests on two subjects.

(b) Each point represents the mean (±SE) of two tests on each of two subjects.

Figure 3.– Least-squares curves of sequential measurements of I^{131} and Evans Blue spaces.

Interindividual variability in plasma volume was measured in 13 subjects with I^{131} and 12 subjects with Evans Blue (table 4); some subjects were used for both measurements. The mean (±SE) for the I^{131} space were 3,335 (±155) ml, 46.1 (±1.84) ml per kilogram body weight and 1.78 (±0.07) liter/m^2 of body surface; the means (±SE) for Evans Blue space were 3,216 (±146) ml, 41.2 (±1.55) ml per kilogram of body weight, and 1.63 ±0.06 liter/m^2 of body surface. Average intraindividual variability (±SE) was ±98 ml for I^{131} space and ±148 ml for Evans Blue space. The difference in the means of the plasma volume measured by the two methods was not statistically significant.

The plasma volumes, ranging from 2,610 to 4,590 ml (I^{131} method) and from 2,585 to 4,355 ml (Evans Blue method, table 4), were in good agreement with the findings of Crispell, Porter, and Nieset (ref. 11) who reported plasma volumes from 2,232 to 3,860 ml by I^{131} albumin measurement and from 2,120 to 3,800 ml by Evans Blue measurement. Similarly, Myhre, Brown, Hall, and Dill (ref. 17) found that the plasma volumes of normal subjects varied from 2,120 to 3,610 ml (Evans Blue method). Hansen (ref. 18) reported plasma volumes from 2,439 to 3,041 ml

by I^{131} albumin measurement. The mean Evans Blue space of the 12 subjects in this study was 3,216 ±146 ml (table 4), which compares favorably with the 3,106 ±402 ml found by Retzlaff, Tauxe, Kiely, and Stroebel (ref. 19).

Extracellular Fluid Volume

The extracellular fluid space of the body was calculated from the bromide concentration in plasma after equilibration and the quantity excreted in urine. It should be stressed that the bromide space probably measures a volume greater than the true extracellular fluid volume because bromide is incompletely removed from the alimentary tract (2 to 3 percent residual) and because it is not entirely an extracellular ion, for example, it is present in red blood cells (approximately 3 to 5 percent).

The distribution of the bromide ion in the body 8 hours after administration is similar to that of the chloride ion distribution, except that bromide slowly penetrates the central nervous system. The ratio of BR^{82} to Cl in the cerebral spinal fluid is only about a third of that in plasma (ref. 3). Although bromide and chloride ions are halogens, the various cells handle them differently. The BR^{82} space is about 2 percent higher than the Cl^{38} space 2 1/2 hours after the isotopes are given intravenously, even though there is a greater concentration of BR^{82} than Cl^{38} in the upper part of the alimentary tract and in the saliva (ref. 20). On the contrary, the kidney excretes chloride in preference to bromide (ref. 21). These findings suggest that bromide and chloride are not completely interchangeable when used for measuring ECF volume.

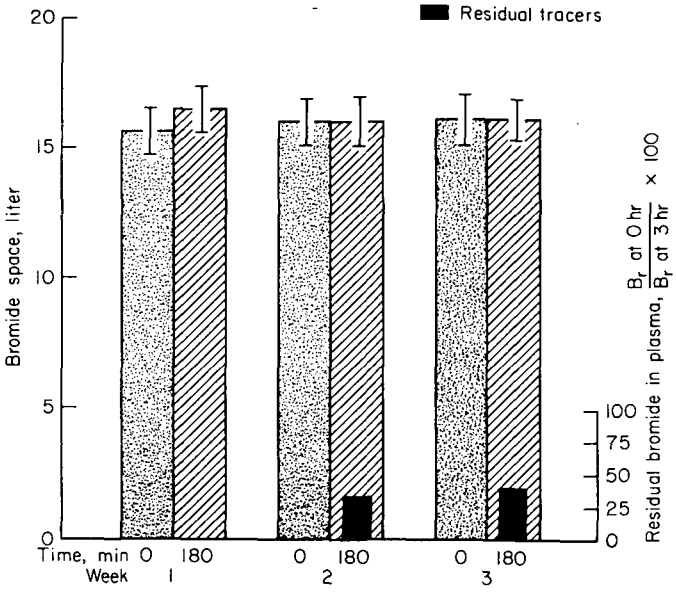


Figure 4.– Comparison of average bromide spaces in seven subjects measured in three consecutive weeks between the 0-min extrapolation value and that computed from the 180-min sample. The solid bars at the bottom represent the residual bromide (expressed as a percent of the total bromide after ingestion).

In the present study bromide space was measured by zero time extrapolation and from the single determination of the 3-hr sample. The mean values of extracellular fluid space determined by both methods (0 min vs. 180 min) were essentially constant (fig. 4). The mean (±SE) extracellular fluid volumes were 15.6 (±0.20) liters by extrapolation and 15.8 (±0.17) liters for the 180-min measurement (NS). The consistency of these results indicates that uniform distribution of bromide in the extracellular space was achieved within 3 hours.

The effect of bromide buildup on consecutive extracellular volume measurements was measured in seven subjects once each week for three consecutive weeks (table 5). (Bromide retained in

TABLE 5.– RESIDUAL BROMIDE AND THE BROMIDE SPACE IN REPEATED MEASUREMENTS 3 HOURS AFTER INGESTION

Subject	Residual bromide $[Br]_0$* (mEq/liter)	Ingested dose $[Br]_3 - [Br]_0$ (mEq/liter)	$\frac{[Br]_0(100)}{[Br]_3 - [Br]_0}$ (percent)	Bromide space		
				liter	liter/kg	liter/m^2
Week 1						
CH	0.0	1.33	0.0	17.00	0.21	8.34
TS	.0	1.43	.0	15.78	.22	8.62
JRo	.0	1.44	.0	16.00	.20	7.58
VC	.0	1.50	.0	15.25	.21	8.20
KN	.0	1.40	.0	15.03	.21	8.08
JRe	.0	1.35	.0	16.92	.20	8.13
TV	.0	1.49	.0	15.52	.22	8.53
Mean	.0	1.42	.0	15.94	.21	8.21
±SE	.0	.02	.0	.30	.00	.13
Week 2						
CH	.65	1.38	47.1	16.60	.21	8.10
TS	.70	1.48	47.3	15.30	.21	8.36
JRo	.70	1.40	50.0	16.12	.20	7.64
VC	.79	1.55	51.0	14.60	.20	7.85
KN	.70	1.59	44.0	14.50	.21	7.80
JRe	.68	1.37	49.6	—	—	7.96
TV	.49	1.47	33.3	15.20	.22	8.35
Mean	.67	1.46	46.0	15.39	.21	8.01
±SE	.03	.03	2.3	.34	.00	.10
Week 3						
CH	1.08	1.28	84.4	17.40	.22	8.49
TS	.66	1.47	44.9	15.60	.22	8.52
JRo	1.05	1.46	71.9	15.70	.20	7.44
VC	1.18	1.52	77.6	14.90	.21	8.01
KN	.83	1.54	52.6	14.90	.21	8.01
JRe	.75	1.42	51.4	16.20	.19	7.79
TV	.75	1.45	51.7	15.70	.22	8.63
Mean	.89	1.45	62.1	15.77	.21	8.13
±SE	.08	.03	5.9	.32	.01	.17

*$[Br]_0$ is the plasma bromide concentration before bromide admistration; $[Br]_3$ is the plasma bromide concentration 3 hours after bromide admistration: Bromide space was determined from the 3-hr bromide concentration in plasma.

the body was expressed as a percent of the injected dose.) The buildup of plasma bromide was 46 percent of the ingested dose in week 2 and 62.1 percent of the ingested dose in week 3. In week 3, the residual bromide level was about 39 percent of the total plasma bromide concentration. This buildup of bromide in the plasma had no effect on the measurement of bromide space in three weekly determinations (fig. 4, table 5).

The average total bromide concentration in the body in the third week, including the residual bromide, ranged from 2.15 to 2.70 mEq/liter, well within the accepted tolerance level of 13 mEq/liter or 1 gm/liter. These results indicate that bromide space can be measured accurately in three consecutive weekly determinations.

Interindividual variability of bromide space in seven subjects ranged from 14.8 to 17.0 liters. The means (±SE) were 15.8 (±0.31) liters, 0.208 (±0.004) liter per kilogram body weight, and 8.12 (±0.13) liters/m^2 body surface (fig. 4). Average intraindividual variability (±SE) was ±170 ml. These

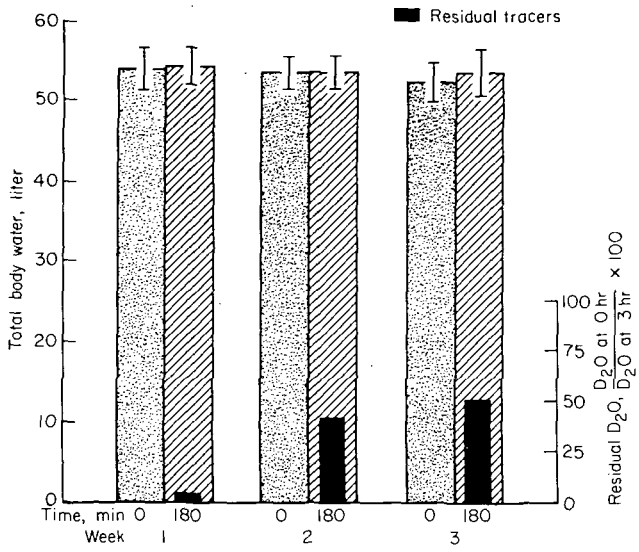


Figure 5.– Comparison of average deuterium oxide spaces in seven subjects measured in three consecutive weeks between the 0-min extrapolation value and that computed from the 180-min sample. The solid bars at the bottom represent the residual deuterium (expressed as a percent of the total deuterium after ingestion).

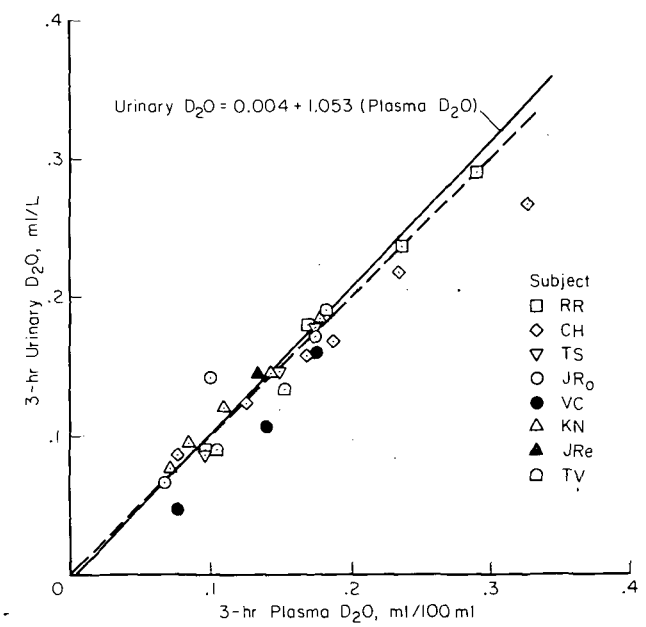


Figure 6.– Regression of the deuterium oxide concentrations in plasma and urine in the 3-hr postingestion samples in eight subjects. The solid line was fitted by the method of least squares and the regression equation is urinary $D_2O = 0.004 + 1.053$ (plasma D_2O). The dotted line is the line of identity.

values for bromide space were higher than the mean sulfate space on 11 healthy males (13.4 (±1.5) liters and 0.191 (±1.7) liters per kilogram body weight) reported by Ryan, Pascal, Inoye, and Bernstein (ref. 22); however, they are lower than the mean bromide space of three laboratory workers (18.6 liters and 0.252 liter per kilogram body weight) reported by Cheek (ref. 15). There is no apparent explanation for the relatively low values of this study since an average of 95 percent bromide added in vitro into plasma was recovered. This suggests that the values found in this study are possibly closer to the actual extracellular space.

Total body water – D_2O space was calculated from the 180-min plasma concentration and by extrapolating to zero time the 2-, 3-, 5-, and 8-hr plasma samples. The mean D_2O space for the seven subjects for the average of the three measurements taken one week apart was 54.1 liters for the 0-min extrapolation calculation and 54.0 liters for the 180-min calculation (fig. 5). Reliable measurements of D_2O space using one 180-min sample depend on total equilibration of D_2O with total body water within 3 hours; the consistent plasma and urinary D_2O concentrations confirm equilibration at 3 hours (fig. 6). These results indicate that D_2O space determined from one 180-min sample is as accurate as the zero time extrapolation.

The effect of D_2O buildup on consecutive total body water measurements is presented in figure 5. In week 3, the residual D_2O concentration was 104 percent of the following ingested dose and 50 percent of the total D_2O concentration in plasma after ingestion of the new dose, but this high background had no significant effect on the measurement of D_2O space (table 6). Both 0-min and 180-min levels were essentially constant over the three weeks. Thus, there is no significant

TABLE 6.– RESIDUAL D_2O AND TOTAL BODY WATER SPACE IN REPEATED MEASUREMENTS 3 HOURS AFTER INGESTION

Subject	Residual $[D_2O]_0$ (ml/100 ml)	Ingested dose $[D_2O]_3 - [D_2O]_0$ (ml/100 ml)	$\frac{[D_2O]_0 \times 100}{[D_2O]_3 - [D_2O]_0}$ (percent)	Total body water space		
				liter	percent body weight	liters/m^2
Week 1						
CH	0.002	0.075	2.67	58.91	73.36	28.74
TS	– .006	.090	6.67	49.64	69.42	27.13
JRo	.008	.060	13.33	58.24	71.72	27.60
VC	.000	.070	.00	56.73	79.01	30.50
KN	.006	.078	7.69	50.91	72.41	27.37
JRe	.000	.071	.00	61.26	70.98	29.45
TV	.008	.097	8.25	44.65	63.33	24.53
Mean	.003	.077	5.52	54.33	71.46	27.90
±SE	.002	.005	1.85	2.28	1.77	.73
Week 2						
CH	.051	.075	68.00	57.28	71.33	27.94
TS	.064	.084	76.19	50.87	71.14	27.80
JRo	.058	.041	141.46	-	-	-
VC	.060	.079	75.95	55.07	76.69	29.61
KN	.056	.087	64.37	50.76	72.20	27.29
JRe	.039	.072	54.17	60.91	70.57	29.28
TV	.060	.092	65.22	46.25	65.60	25.41
Mean	.055	.076	77.91	53.52	71.26	27.89
±SE	.003	.006	10.97	2.15	1.45	.62
Week 3						
CH	.088	.081	108.64	53.23	66.28	25.97
TS	.086	.089	96.63	49.23	68.93	26.93
JRo	.096	.079	121.52	55.53	68.38	26.32
VC	.098	.077	106.52	56.18	78.24	30.20
KN	.086	.092	93.48	47.25	67.21	25.40
JRe	.069	.064	107.81	67.59	78.31	32.50
TV	.088	.094	93.62	46.20	65.53	25.38
Mean	.087	.082	104.03	53.60	70.41	27.53
±SE	.004	.004	3.85	2.76	2.08	1.04

interference for the measurement of D_2O space at weekly intervals even though there was a 50 percent buildup of residual deuterium oxide. Note that the clearance rate of D_2O in our subjects varied from 3.7 to 6.3 percent per day (table 7).

Interindividual variability of total body water in seven subjects ranged from 45.7 to 63.3 liters, and the mean D_2O spaces (±SE) were 54.1 (±2.3) liters, 71.2 (±4.0) liters per kilogram body weight, and 27.9 (±0.7) liters/m^2 body surface (fig. 5). Average intraindividual variability (±SE) was ±0.87 liter. The results of this study agree with those of Moore (ref. 1) who found a mean D_2O space of 47.8 liters (72% of body weight) in normal adults from plasma samples taken 2 hours after intravenous injection of D_2O. Other investigators (refs. 23–26) have

TABLE 7.– D_2O CLEARANCE RATE

Subject	Duration between measurements (day)	Clearance rate (percent per day)	
			Mean
RR	2	5.0, 5.5, 8.3	6.3
CH	7	4.9, 4.5	4.7
CH	9	5.1	5.1
CH	2	6.6, 4.4	5.5
TS	7	4.7, 6.0	5.4
VC	7	3.2, 4.2	3.7
KN	7	4.7, 5.6	5.2
JRe	7	6.3, 5.1	5.7
TV	7	6.1, 6.0	6.1

reported lower normal values for total body water. For instance, Edelman and co-workers (ref. 24) found mean total body water levels of 44.1 liters, 61.1% of body weight, and 23.3 liters/m^2. One possible explanation for our apparently higher values compared with those of other investigators is that most of our subjects were athletic young men who probably had greater lean body masses and consequently greater total water content. Pascale and co-workers (ref. 27) reported that after three weeks of paratrooper training, the mean intracellular water and total body water volumes of 12 men increased 5 to 7 percent. It appears that there is an increase in the water content of muscles induced by physical training (ref. 28).

CONCLUSIONS

The purpose of this study was to compare the use of Evans Blue with I^{131} for measuring plasma volume and to determine the reproducibility of bromide and deuterium oxide for measuring extracellular fluid volume and total body water volume, respectively. From these results the following conclusions are drawn:

(a) Both Evans Blue space and I^{131} space, determined from a single sample obtained 10 min after injection, were comparable with the zero time extrapolation values determined from serial sampling.

(b) A total of four injections of I^{131} on alternate days, resulting in the residual radiation buildup of 85 percent of one dose, does not significantly affect the accuracy of measurement of I^{131} space.

(c) A total of four injections of Evans Blue on alternate days, resulting in the residual dye buildup of 87 percent of one dose, does not significantly affect the accuracy of measurement of Evans Blue space.

(d) Evans Blue and I^{131} do not interfere with each other when the two tracers are used interchangeably for determining plasma volume and the results for both are comparable.

(e) Average interindividual variability (±SE) was ±155 ml for I^{131} space and ±146 ml for Evans Blue space; average intraindividual variability (±SE) was ±98 ml for I^{131} space and ±148 ml for Evans Blue space.

(f) Both bromide space and deuterium oxide space, determined from a single sample obtained 180 min after the tracer was ingested, were comparable with the zero time extrapolation values determined from serial sampling.

(g) The ingestion of sodium bromide once each week for three consecutive weeks, resulting in the residual tracer buildup of 46 percent of one dose, does not significantly affect the accuracy of measurement of bromide space.

(h) Average interindividual variability (±SE) in bromide space was ±310 ml; average intraindividual variability (±SE) was ±170 ml.

(i) Ingestion of deuterium oxide once each week for three consecutive weeks, resulting in the residual tracer buildup of 104 percent of one dose, does not significantly affect the accuracy of measurement of D_2O space.

(j) Average interindividual variability (±SE) in total body water was ±2300 ml; average intraindividual variability (±SE) was ±870 ml.

Ames Research Center
National Aeronautics and Space Administration
Moffett Field, Calif., 94035, Oct. 25, 1972

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